Chemotherapy of Cancer: *

Regional Perfusion Utilizing an Extracorporeal Circuit

OSCAR CREECH, JR., M.D., E. T. KREMENTZ, M.D., ROBERT F. RYAN, M.D., JAMES N. WINBLAD, M.D.

From the Department of Surgery, Tulane University School of Medicine and the Tulane Surgical Services of Charity Hospital and Touro Infirmary, New Orleans, La.

CHEMOTHERAPY of cancer began about 15 years ago as a result of studies to determine the biologic action of the mustard compounds.[4-6] These studies revealed that the B-chloroethyl amines and sulfides have a cytotoxic effect on cells unlike that of other chemical agents and closely resembling that of x-rays. Since cellular susceptibility appeared to be related to the degree of proliferative activity these agents were administered to mice with transplanted lymphosarcoma and it was noted that the tumors rapidly disappeared. However, the dose required approached the toxic level and the tumors invariably recurred. Subsequent clinical trial in about 150 cases consisting of Hodgkin's disease, lymphoma, and leukemia resulted in remissions in a majority of patients with Hodgkin's disease but relatively little effect was observed in those with leukemia and lymphosarcoma. It was the concensus then that "the therapeutic efficacy of the nitrogen mustards is no greater than that of x-rays." [5]

The limiting factor in the use of these agents is the toxic effect on normal tissues, particularly the hemopoietic system and the gastro-intestinal tract.[3] Thus, administration of doses minimally effective against malignant tumors is followed by temporary depression of hemopoiesis and transient gastro-intestinal disturbances, while larger doses are hazardous to life because of severe involvement of these two systems.

In an attempt to avoid the systemic toxic effects and at the same time increase the dose of nitrogen mustard, Klopp and associates [10] developed technics for the intra-arterial administration of this compound. A cannula was placed into the major artery supplying a tumor-bearing site and nitrogen mustard administered in single, or fractionated doses. In some instances venous occlusion was produced temporarily to confine the agent to the involved part for the duration of its action. It was observed that the hematologic consequences were the same following intravenous (systemic) and intra-arterial administration, but when venous occlusion was combined with fractionated intra-arterial administration the hematologic changes were less pronounced.[1] More recently Klopp [9] has reported the intra-arterial use of nitrogen mustard as an adjunct to radiation therapy.

In spite of increased effectiveness of the mustards when administered directly into an artery supplying a tumor, the limiting factor of systemic toxicity still precludes the use of these agents in amounts large enough to completely irradicate malignant neoplasms. Thus, in spite of the proven cytotoxic action of the alkylating agents no method has been available for exposing malignant tumors to maximally effective concentrations of these drugs without, at

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the same time, risking irreparable damage to the hemopoietic and gastro-intestinal systems.

Recently, as a result of experiences with a heart-lung apparatus in the treatment of intra-cardiac defects, it occurred to us that this extracorporeal circuit might provide a means of temporarily isolating and maintaining a tumor-bearing area while it was being perfused with maximal amounts of an alkylating agent. If vascular and lymphatic exclusion were complete systemic toxic effects should be eliminated while the specific activity of the agents would be brought to bear only upon the tumor and its immediate environment. Since these agents have a radiomimetic effect and since ionizing radiation is potentiated by high oxygen tension in the tissues [2, 7] it was postulated that incorporation of a bubble oxygenator into the circuit, thus producing arterial oxygen tensions of 500 to 600 mm./Hg, might increase the effectiveness of the mustard compounds. This report is concerned with the preliminary experimental development and subsequent clinical application of the technics of selective perfusion.

Experimental Studies

Experiments were designed to investigate the following: 1) the extent to which certain vascular beds could be isolated from the systemic circulation, 2) the effects of perfusion of oxygenated blood alone and with chemotherapeutic agents added on these isolated areas, 3) the maximum amount of these agents that can be used safely in perfusion, and 4) the duration of action of nitrogen mustard and phenylalanine mustard (PAM).* The details of these experiments have been reported in part elsewhere.[12, 13] Essentially, it was demonstrated that the limbs, mid-gut, and liver of the dog can be excluded from the systemic circulation and perfused at moderate flow rates for about 30 minutes without producing any undesirable effect either in the perfused area or systemically. At maximal flow rates or when perfusion was continued longer than 30 minutes edema of the perfused tissue occurred. Careful control of perfusing pressure appeared to be particularly important in experiments on the mid-gut and liver.

To compare the maximum tolerable dose of the mustards administered intra-arterially and by perfusion, varying amounts of these agents were injected directly into the femoral artery. It was observed that 0.5 to 0.6 mg./Kg. of HN_2 produced arterial thrombosis with necrosis of the limb. These animals did not survive long enough to demonstrate hematologic changes. On the other hand, utilizing perfusion technics 0.8 to 1.0 mg./Kg. was well tolerated. Phenylalanine mustard could be safely administered directly into the femoral artery in a dose of 1.0 mg./Kg. while by perfusion 2.0 mg./Kg., the recommended dose for oral administration, produced no ill effects. Thus, HN_2 can be administered by perfusion to the isolated hind limb of a dog in a dose twice that which can be safely administered systemically, while the usual systemic dose of PAM is well tolerated in the isolated limb. Since this extremity of the dog constitutes $\frac{1}{20}$ of the total body weight the concentration of HN_2 and PAM supplied by perfusion to the isolated limb is far in excess of the concentration achieved by intravenous or direct intra-arterial administration of the recommended doses.

The maximum safe doses of HN_2 and PAM when administered by perfusion into the mid-gut and liver have not been determined although the amounts generally employed for systemic administration, namely, 0.4 mg./Kg. and 2.0 mg./Kg. respectively, are well tolerated by these isolated viscera.

To determine the completeness of isolation of the various areas, blue dye (T-1824)

* Nitrogen mustard supplied through the courtesy of Merck, Sharp & Dohme, Philadelphia, Pa.
PAM supplied through the courtesy of Dr. A. Clark Griffin, Anderson Hospital, Houston, Texas.

TABLE 1. *Types of Malignant Neoplasms Treated with NH_2 and PAM Administered by Perfusion*

Malignant melanoma		6
Sarcoma		9
Rhabdomyosarcoma	2	
Osteogenic	2	
Chondrosarcoma	1	
Synovioma	1	
Alveolar cell	1	
Liposarcoma	1	
Fibrosarcoma	1	
Carcinoma		9
Adenocarcinoma	6	
Epidermoid	2	
Undifferentiated	1	
Total		24

was injected into the perfusing system and samples were drawn periodically from a systemic vein. It was demonstrated that the technics developed for perfusion of the limbs, mid-gut and liver effectively separate the systemic from the perfused system.

The duration of action of the mustard compounds was determined by bioassay studies. The hind limb of the dog was isolated and perfused with 0.8 mg./Kg. of nitrogen mustard administered in three equal amounts six minutes apart. Samples of blood were removed from the venous side of the perfusion system three minutes following the first dose, three and five minutes following the second, and four and six minutes after the third dose. Blood samples were injected into the peritoneal cavity of mice in amounts of 0.5, 1.0 and 2.0 ml. and the LD_{50} determined. Similar experiments were performed using phenylalanine mustard, 2.0 mg./Kg. being administered in two equal doses five minutes apart. Utilizing the LD_{50} as an index of activity of the alkylating agents it was observed that HN_2 retains its activity for about eight minutes following mixing with whole blood while PAM remains active for about two hours. These experiments demonstrate, therefore, that administration of the mustard compounds in divided doses by perfusion results in high concentrations of these agents in the perfusate which last for five minutes following the final dose of HN_2 and for at least two hours after the final dose of PAM.

Clinical Studies

Although it was shown experimentally that certain areas could be isolated and safely perfused with oxygenated blood containing high concentrations of the mustard compounds without producing systemic toxic effects, it was necessary to employ these technics in the treatment of human tumors to determine their therapeutic efficacy. Accordingly, 24 patients with a variety of malignant neoplasms have been treated by these methods with nitrogen or phenylalanine mustard (Table 1). There were six patients with metastatic melanoma treated by perfusion with phenylalanine mustard. In each the lesions involved the lower extremity and represented cutaneous and nodal metastases following excision of the primary tumor. Groin dissection had been performed prior to perfusion in four patients and was performed simultaneously with or subsequent to perfusion in the remaining two patients. In four instances the metastatic lesions were so distributed that hind-quarter amputation might have been feasible but none of the patients would accept amputation. In two other cases the tumor had extended beyond the limb.

There were nine patients with sarcomas treated by perfusion, HN_2 being administered in seven and PAM in two. The lesions were resected subsequent to perfusion in six patients and in three post-perfusion resection was recommended but refused.

Nitrogen mustard was administered to nine patients with carcinomas involving the following structures: transverse colon, thumb, uterus, lung, breast, rectum and scapula. In two perfusion was employed as an adjunct to excision of a localized pri-

mary lesion while in the remainder therapy was undertaken for palliation only.

The technics of perfusion employed in the clinical cases were essentially those developed in the experimental animal, although certain modifications were necessary. The extracorporeal circuit consisted of a bubble oxygenator and a Sigmamotor pump. For perfusion of the limbs, breast, pelvis and lungs a disposable oxygenator was used which had a reservoir capacity of about 500 ml., and an oxygenating capacity of about 4000 ml. per minute (Fig. 1).* Utilizing 100 per cent oxygen the blood was completely saturated at a PO_2 of 500 to 600 mm. Hg. In lung perfusion it was necessary to employ a systemic extracorporeal circuit also, and for this purpose a DeWall, helix-reservoir type of bubble oxygenator was used. The mustard compounds were injected through the plastic tubing proximal to the arterial pump to insure thorough mixing of the agent with the blood and to minimize dilution.

The artery and vein supplying the tumor-bearing area were isolated and cannulas of appropriate size inserted. For the lower extremity the common femoral artery and vein were cannulated. In lesions of the arm and forearm the axillary artery and vein were utilized and for tumors of the shoulder perfusion was accomplished by way of the subclavian vessels. The subclavian artery and vein were exposed by resection of the medial half of the clavical or by median sternotomy. For pelvic tumors the aorta and inferior vena cava were occluded below their renal branches and cannulated just above their bifurcation. Pneumatic tourniquets were applied to both limbs. Lung perfusion was accomplished through a left atrium-to-pump oxygenator-to-pulmonary artery circuit while the systemic circulation was maintained with the standard cardio-pulmonary bypass consisting of

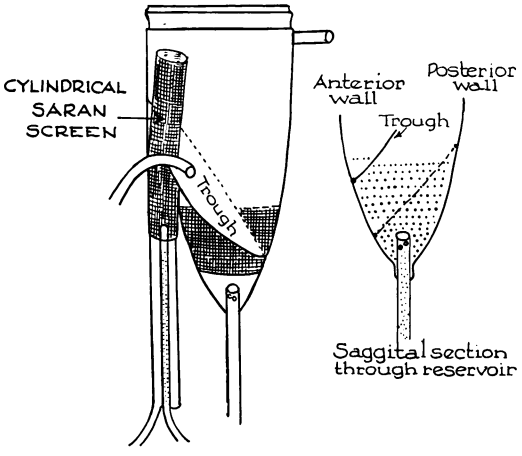

FIG. 1. Diagrammatic sketch of small, disposable oxygenator used in perfusion studies.

venae cavae-to-pump oxygenator-to-femoral artery circuit. Complete isolation of the extremities was accomplished by applying an Esmarch tourniquet proximal to the cannulas. In perfusion of lung tumors, the use of two circuits and caval occlusion prevented mixing between the systemic and pulmonic circuits. However, in the case of pelvic, breast and shoulder perfusions tourniquets would have been ineffective and the negative pressure produced in the venous circuit by the Sigmamotor pump was relied upon to minimize systemic mixing.

Upon completion of perfusion with nitrogen mustard the tourniquet was released, the cannulas removed and the arterial and venous incisions repaired. When phenylalanine mustard was employed the perfused area was drained with the venous pump in order to remove the still-active agent.

In one case sodium, potassium, chloride, pH, arterial and venous oxygen saturation and urea nitrogen were measured in blood samples taken from the systemic and isolated circuits before, during and after perfusion.

Following operation daily erythrocyte, leucocyte and platelet counts were performed. Whenever possible the neoplasms were biopsied 24 hours after operation and

* This oxygenator was designed by Dr. Edward Hyman and manufactured by Abbott Laboratories.

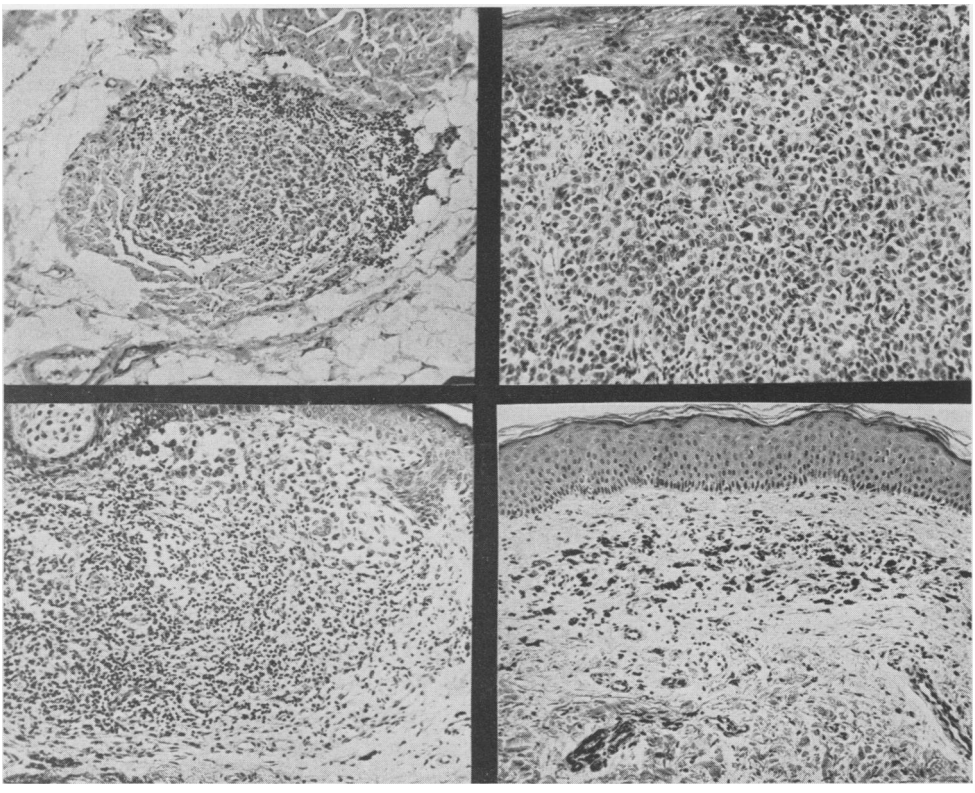

FIG. 2. (Case 1.) Malignant melanoma in tissue removed at groin dissection is shown in (a) upper left; comparison of cutaneous metastases (b) upper right before, and (c lower left and d lower right) after perfusion reveals striking regression of the lesions.

at varying intervals thereafter to observe any changes that might occur.

In the following case reports the technics of perfusion of the limbs, pelvis and lungs for malignant neoplasms are described in detail and some of the problems inherent in this type of therapy are presented.

Case Reports

Case 1. J. S. P., a 76-year-old white man, was admitted to the New Orleans Charity Hospital on April 20, 1956, complaining that a small black mole on the dorsum of the left foot, which had been previously asymptomatic, had recently become irritated and bled. One week following admission the lesion was widely excised and the area covered with a skin graft. Upon microscopic examination it proved to be a malignant melanoma. On June 1, 1956, the patient was re-admitted to the hospital, and a left groin dissection was performed. There was no evidence of involvement of the excised lymph nodes, but tumor cells were noted in a small lymphatic (Fig. 2a).

The patient was asymptomatic until May 1957, when he noted the appearance of many pigmented lesions on the medial aspect of the left leg and thigh (Fig. 3a). On May 16, biopsy of one of these lesions revealed them to be metastatic melanomas (Fig. 2b). He was re-admitted to the hospital and on June 11, perfusion with PAM was performed. Under spinal anesthesia an oblique incision was made in the left groin and the common femoral artery and vein were isolated. The patient was heparinized with 2 mg./Kg. of heparin and the common femoral artery and vein were cannulated with plastic catheters 3/16 inch i.d. (Fig. 4). The cannulas were connected to an extracorporeal circuit consisting of a miniature DeWall-type bubble-oxygenator with a reservoir capacity of about 500 ml. Oxygenation of venous blood was accomplished with 100% oxygen at a flow rate of 5 L/minute. An Esmarch tourniquet previously placed in the gluteal crease and over the left iliac crest was tightened to produce venous occlusion and perfusion was begun. A maximal flow rate was achieved within about 2 minutes and at this time the first of 4 equal doses of PAM was injected into

the afferent limb of the arterial pump. The dose of PAM was based on a schedule of 2 mg./Kg. or a total of 120 mg. divided into 4 doses of 30.0 mg. each administered at 5 minute intervals. When the last dose of PAM was administered 5 cc. of Evans Blue Dye was injected into the arterial line; subsequent samples were withdrawn simultaneously from a systemic vein and from the venous limb of the extracorporeal circuit. The duration of perfusion was 23 minutes. At the end of this time the tourniquet was released, the cannulas removed and the artery and vein repaired.

The patient's course after operation was uneventful (Chart 1). There was no change in the normal appearance of the extremity and no edema was evident at any time. Periodic erythrocyte, leucocyte and platelet counts revealed no evidence of hemotologic damage. Cutaneous metastases were biopsied on the 2nd, 8th, 30th, 50th and 77th day respectively after operation. Within 3 weeks it was apparent grossly that the metastases were undergoing a significant change. Originally there were about 175 raised, dark red metastatic lesions on the extremity. After perfusion the lesions became flattened and black; then a superficial crust formed over their surface, the pigment began to fade and finally the crust fell away leaving a light "freckle" in place of the metastasis.

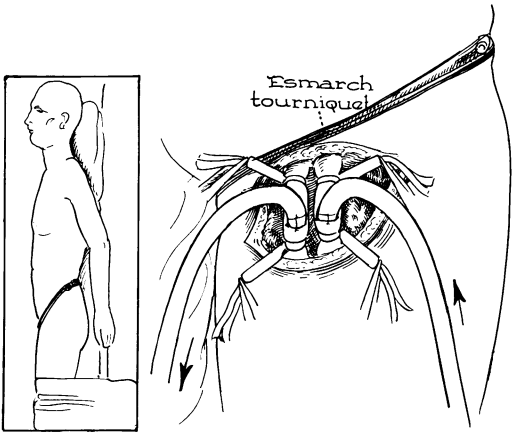

FIG. 4. Technic of cannulation of femoral artery and vein and application of an Esmarch tourniquet for perfusion of a lower extremity. A Steinman pen is inserted through the iliac crest to maintain the tourniquet at a proper level.

FIG. 3. Photographs of cutaneous metastases in Case 1, (a) upper before, and (b) lower after perfusion.

In order to determine the histologic changes characterizing these alterations in gross appearance biopsies were taken of lesions at the stages described above. These revealed an initial lymphocytic and round cell invasion of the neoplasm followed by necrosis of tumor cells and fibrous tissue replacement and finally complete disappearance of tumor cells (Fig. 2c, d).

This patient has been followed for almost a year since perfusion. During this time the metastatic lesions have continued to regress and disappear (Fig. 3b). Examination in April 1958 disclosed only 8 lesions remaining and these are flattened and dark. No new lesions have appeared since perfusion.

Comment: Because of his age and the extensiveness of the cutaneous metastases, this patient was not a candidate for hindquarter amputation. Therefore, perfusion of the involved extremity with PAM was undertaken for palliation only.

The blue dye studies demonstrated that the left lower extremity was completely iso-

lated from the systemic circulation. Subsequent to this case, it was demonstrated that PAM remains active in blood for about two hours; therefore the extremity should be drained of blood contaminated with the agent prior to release of the tourniquet if systemic toxic effects are to be avoided.

Case 2. B. P., an 18-year-old girl, was admitted to Charity Hospital on January 24, 1957, with the history of a mass on the left forearm of about 2 weeks duration. On February 27, this lesion was excised and was found to involve the flexor digitorum sublimis muscle to the left index finger. Pathologic examination revealed rhabdomyosarcoma; therefore, on April 25, a supracondylar amputation was performed.

The patient was re-admitted to Charity Hospital on August 19, with a painful amputation stump and a large mass in the left axilla. Examination disclosed recurrent tumor in the amputation stump, this lesion measuring about 10×8 cm., and an axillary metastasis about 10 cm. in diameter (Fig. 5a, b). Forequarter amputation was recommended but the patient refused any additional ablative procedure. Therefore, on October 4, perfusion with HN_2 was accomplished. With the patient in the supine position median sternotomy was performed and through this incision the left subclavian artery and left inominate vein were isolated (Fig. 6). Plastic cannulas, $\frac{3}{16}$ inch i.d. were placed into these vessels and directed distally. The subclavian artery and left inominate vein were then temporarily occluded proximal to the

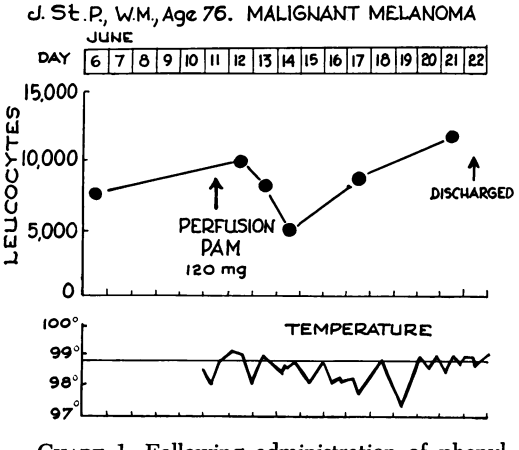

CHART 1. Following administration of phenylalanine mustard to the left lower extremity by perfusion there was no significant change in the leucocyte count indicating that action of the agent was confined to the extremity.

FIGURE 5a

FIG. 5. Recurrence of rhabdomyosarcoma in an amputation stump and metastases to the axilla in Case 2 are shown in (a). Roentgenogram revealed extensive involvement of the lower third of the arm (b).

sites of cannulation and the cannulas were connected to an extracorporeal circuit consisting of a bubble oxygenator and Sigmamotor pump. When a balanced flow through the circuit was achieved HN_2 was administered in a dose of 0.4 mg./Kg. body weight given in 4 equal amounts 5 minutes apart. The total dose was 30.8 mg. Perfusion lasted 25 minutes and the flow rate was about 400 to 500 ml. per minute. Although a tourniquet had not been employed because of the large axillary mass, blue dye was injected into the perfusion circuit 5 minutes before completion of the procedure in order to determine the degree to which the upper extremity had been isolated. At completion of perfusion the cannulas were removed, the incision in the subclavian artery and left innominate vein repaired and the thoracic wound closed.

Needle biopsies had been taken from the recurrent lesion in the amputation stump and the metastasis in the left axilla prior to operation and biopsy was repeated on the 2nd, 5th, and 30th days after operation. Microscopically, there was evidence of extensive coagulation necrosis of the tumor although viable nests of neoplastic cells were still evident (Fig. 7a, b). Pain in the amputation stump had been severe enough to require opiates prior to perfusion but completely disappeared after perfusion. In addition there was rapid and almost complete disappearance of the axillary mass and the lesion in the amputation stump. On November 5, one month after perfusion, only a

small nodule remained in the left axilla and a roentgenogram revealed that the recurrence at the distal end of the arm had completely disappeared (Fig. 8a, b). At this time forequarter amputation was again recommended but the patient refused and chose to return home. About 2 months later she returned complaining of pain in the sacrum and roentgenograms revealed on osteolytic lesion of the right lumbosacral area. The lesion was treated with irradiation and the patient was also given a course of nitrogen mustard intravenously. Subsequently, additional metastatic lesions have developed in other parts of the skeleton.

Daily erythrocyte, leucocyte and platelet counts did not reveal any significant depression of these cellular elements after operation, although dye studies indicate that the upper extremity had not been completely isolated. Skin changes characteristic of ionizing radiation appeared in the perfused area by the 10th day after operation (Fig. 8a).

Comment: This lesion was not curable by any criteria. Perfusion was undertaken only for the purpose of determining whether rhabdomyosarcoma, previously believed to be resistant to the mustard compounds and to radiation, would respond to this agent when administered in high concentration. Although there was remarkable regression of the lesions following perfusion, it is unlikely that forequarter amputation would have altered the subsequent course since apparently skeletal metastases were already present. The radio-mimetic effect of the mustard compounds was demonstrated by the skin changes appearing about ten days after perfusion. Although vascular isolation of the tumor-bearing area was not feasible, skin changes were confined to the region in which a maximal concentration of nitrogen mustard was desired.

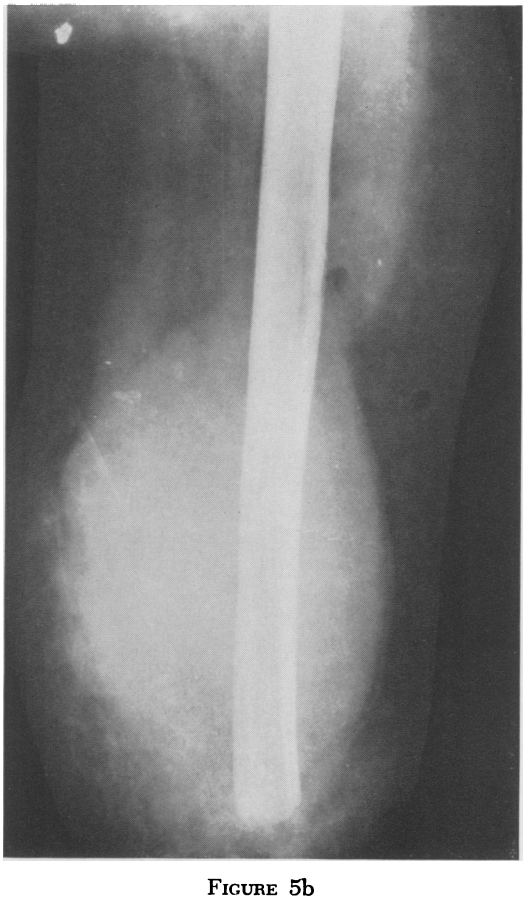

FIGURE 5b

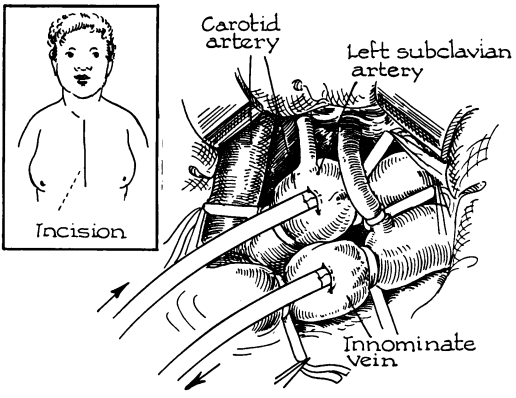

FIG. 6. Technic of cannulation of the left subclavian artery and left innominate vein for perfusion of the arm and axilla in Case 2.

Case 3. A. L., a 61-year-old medical technician, was admitted to Charity Hospital on January 1, 1958, for perfusion of a pelvic mass. In 1946, hysterectomy had been performed for carcinoma of the uterus. The patient had been asymptomatic until 1955, when a metastatic lesion appeared in the right side of the pelvis and the right innominate bone (Fig. 9a). These areas were irradiated in 1955, and again in 1956, but without significant relief of symptoms or alteration in size of the lesions. In 1956, she received a course of thio-TEPA and in August 1957, because of severe, persistent pain in the right hip and right side of the pelvis, thoracic chordotomy was performed. The pelvic lesions continued to increase in size, however, and swelling of the right thigh and groin became severe (Fig. 10a).

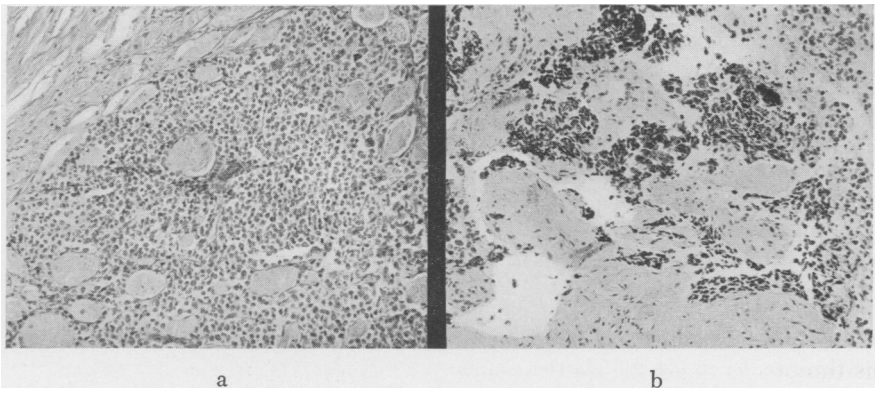

FIG. 7. Photomicrographs of sections removed from recurrent rhabdomyosarcoma in Case 2, (a) left before, and (b) right after perfusion with nitrogen mustard.

On January 8, 1958, perfusion was performed utilizing nitrogen mustard. A midline abdominal incision was made and the lower abdominal aorta and vena cava isolated (Fig. 11). Both of these vessels were occluded just below their renal branches and cannulas 9/32 inch i.d. were inserted in a distal direction and connected to a pump-oxygenator system. Pneumatic tourniquets which had been previously applied to both thighs were inflated to 300 mm./Hg and perfusion was begun. When flow was stabilized at a rate of about 1,000 ml. per minute, the first of four equal doses of nitrogen mustard was injected into the afferent line to the arterial pump. Subsequent doses of HN_2 were administered every 4 minutes for a total dose of 20.4 mg. or 0.4 mg./Kg. body weight.

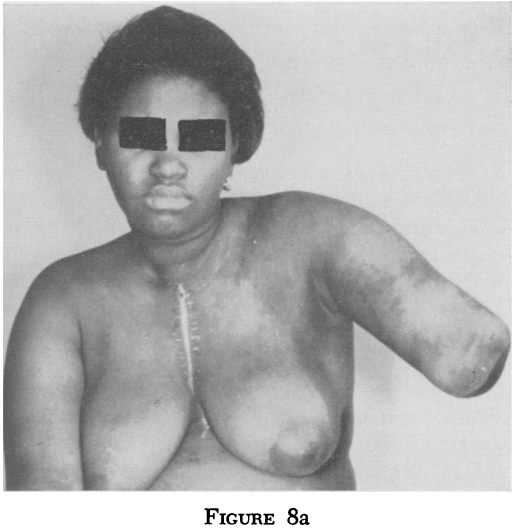

FIGURE 8a

FIG. 8. Following perfusion in Case 2 there was marked regression of the lesions (a and b). Discoloration of the skin of the perfused area is indicative of the radiomemetic effects of HN_2.

During the last 2 minutes of perfusion, Evans Blue Dye was injected into the extracorporeal circuit to determine the completeness of isolation of the involved vascular bed. Perfusion lasted 22 minutes and at the end of this time the cannulas were removed, the incisions in the aorta and vena cava repaired and the abdominal wound closed.

Following operation the pelvic mass decreased in size. The blue dye studies had failed to demonstrate mixing of the systemic and perfusion circuits and there was no alteration in the erythrocyte, leucocyte or platelet counts.

On January 28, the patient was discharged from the hospital and was followed in the outpatient clinic. About 2 months after perfusion, it was noted that the pelvic mass was extremely large, tense and cystic; therefore, it was aspirated and 1,800 ml. of brown fluid mixed with necrotic tissue and old blood was removed. Immediately, the patient exhibited signs of acute hemorrhage and the mass enlarged again and it was evident that bleeding was occurring into the cystic cavity. She was re-admitted to the hospital and after several days the mass was aspirated again and 4.4 mg. of HN_2 injected. This was repeated on a second occasion. On March 21, the cyst, which was now presenting beneath the right buttock, was incised and a large amount of old blood mixed with necrotic tumor removed. The cyst was packed and left open (Fig. 10b). Biopsy of the cyst wall failed to reveal evidence of carcinoma (Fig. 9b).

Comment: Perfusion was carried out in this instance in the hope that palliation might be obtained and to determine the effect of HN_2 on uterine adenocarcinoma. It was also hoped to determine whether vascular isolation of the pelvis was possible. Apparently the negative pressure created in

the venous system draining the pelvis was sufficient to prevent spill into the systemic circulation of blood containing nitrogen mustard.

The response of the large pelvic metastasis was unanticipated. That extensive tumor necrosis occurred following perfusion is indicated by liquefaction of the metastasis and absence of tumor cells in the section removed from the cyst wall. Additional observation should determine the extent to which this tumor has been affected by perfusion with nitrogen mustard.

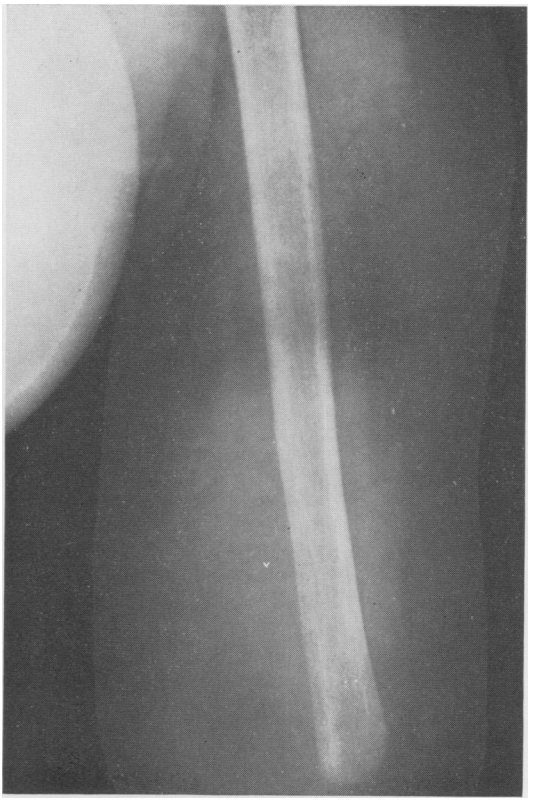

FIGURE 8b

Case 4. D. W., a 54-year-old man, was admitted to Charity Hospital on January 21, 1958, with a six weeks history of respiratory infection. Symptoms had been characterized by fever, a productive cough and weight loss. Physical examination revealed obstructive pneumonitis of the right lung confirmed by roentgenograms of the chest (Fig. 12a). Bronchoscopy disclosed obstruction of the right main bronchus by a neoplasm that extended onto the trachea and biopsy revealed epidermoid carcinoma (Fig. 13). Excision of a lymph node in the right supraclavicular fossa was performed on January 30 but there was no evidence of carcinoma. Thoracentesis yielded a straw-colored fluid with no evidence of malignant cells.

Because of its extent the lesion was considered unresectable and perfusion with HN_2 was performed on February 11. Under endotracheal anesthesia, with the patient in a supine position, bilateral thoracotomy was performed utilizing the 4th intercostal spaces and transection of the sternum (Fig. 14). The epicardium was opened widely, the venae cavae isolated and tapes passed about them. After heparinization, plastic cannulas $\frac{9}{32}$ of an inch i.d. were passed into the venae cavae through the right atrial appendage. The right femoral artery was exposed in the groin and cannulated with a catheter $\frac{3}{16}$ inch i.d. The caval

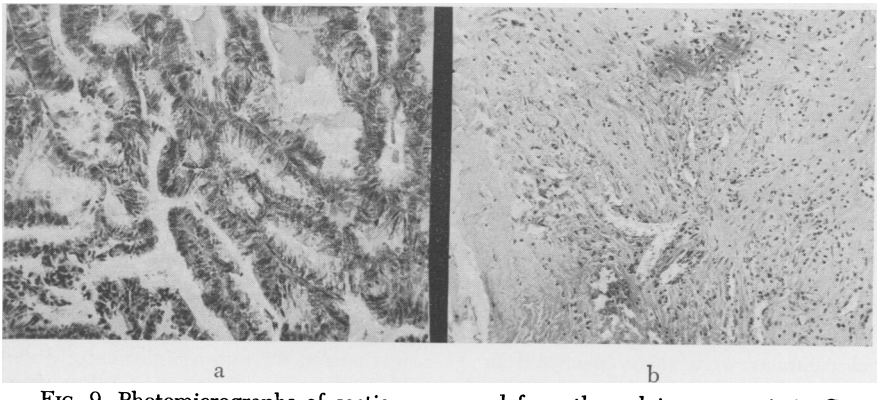

FIG. 9. Photomicrographs of sections removed from the pelvic metastasis in Case 3, (a) prior to perfusion, and (b) after perfusion. In the latter there are no demonstrable neoplastic cells.

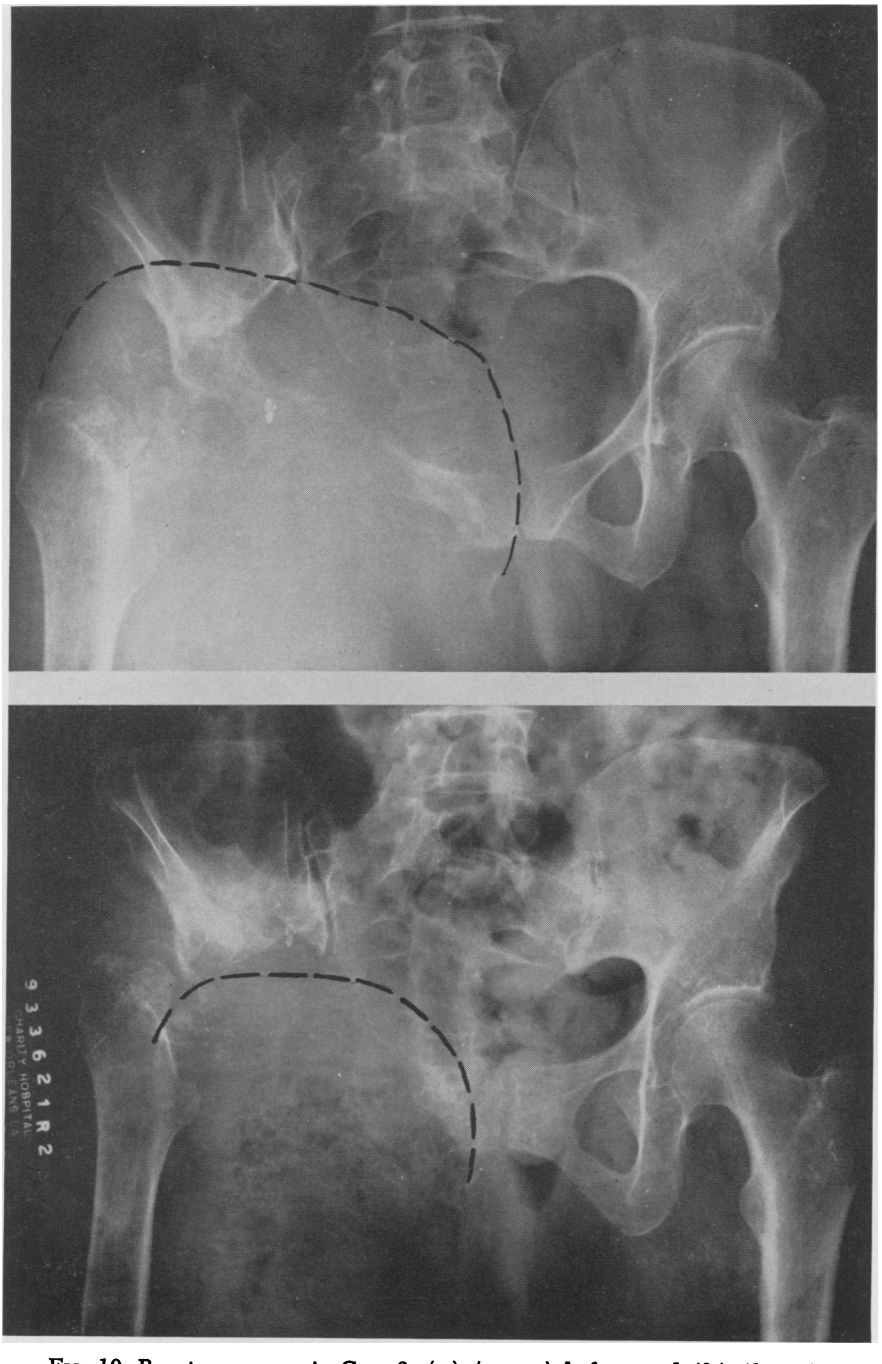

FIG. 10. Roentgenograms in Case 3, (a) (upper) before, and (b) (lower) after perfusion with nitrogen mustard.

and arterial cannulas were then connected to a pump-oxygenator system. Next, a catheter 9/32 inch i.d. and perforated near its tip was placed into the left atrium through the appendage. This in turn was passed through a Sigmamotor pump to a bubble oxygenator. The arterial line from the oxygenator was connected to a catheter 3/16 inch i.d. placed into the right ventricle outflow tract and

through the pulmonic valves into the main pulmonary artery. A tape was passed about the main pulmonary artery so that it could be tightened about the catheter. An additional catheter was inserted through the wall of the right atrium and connected, through a separate pump, to the systemic extracorporeal circuit for pick-up of coronary venous return. The oxygenator of the pulmonary circuit was elevated 40 cm. above heart level for gravity flow into the pulmonary artery. The systemic extracorporeal circuit was placed in operation first. Caval occlusion was accomplished and with coronary sinus drainage diverted the right heart was emptied. Pulmonary perfusion was then begun. The tape about the pulmonary artery was tightend and flow through the venous pump was increased until the left ventricle was empty. When both circuits were stabilized the first of four equal doses of nitrogen mustard was administered into the efferent limb of the pulmonary extracorporeal circuit. Subsequently, at 5 minute intervals 3 additional doses of nitrogen mustard were administered. After 10 minutes of perfusion, Evans Blue Dye was injected into the pulmonary circuit to determine extent of mixing. The duration of perfusion was 30 minutes.

At the completion of perfusion the cannulas were removed and the cardiac and thoracic wounds were closed. The patient's condition after operation was uneventful except for a cough productive of large amounts of mucopurulent material. There was no evidence of hemotologic damage and the temeprature returned to normal by the 5th day. About 7 days after operation breath sounds became audible for the first time over the right lateral chest wall, and there was progressive improvement in the chest roentgenograms (Fig. 12b). The patient has remained asymptomatic since discharge from the hopsital and significant re-expansion of the right lung has taken place.

Comment: This case is of interest primarily because of the technics of perfusion employed rather than for any effect the chemotherapeutic agent may have had upon the neoplasm. The blue dye studies disclosed no spill-over from the pulmonic to the systemic circuit and a gain in volume in the pulmonary circuit of about 100 ml. is assumed to represent bronchial artery flow.

Although it was time-consuming to make the many cannulations necessary to establish the extracorporeal circuits these separate systems appeared to function well

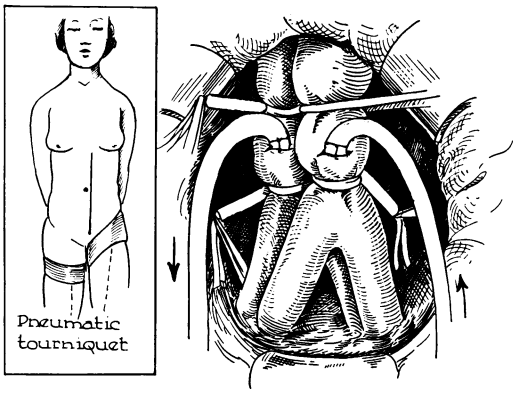

FIG. 11. Technic of cannulation of abdominal aorta and inferior vena cava for perfusion of pelvic structures.

and to have no adverse effect on the patient. With regard to the therapeutic effectiveness of the procedure the patient was not an ideal one since there was complete obstruction of the right main bronchus with collapse of the right lung distal to the lesion. Thus it is questionable whether perfusion of this lung occurred to the same degree as that of the opposite lung. Furthermore, it is doubtful whether a chemotherapeutic agent administered through the pulmonary arterial system reaches maximum concentration within a bronchogenic carcinoma.

Results and Conclusions

In undertaking this study it was postulated that the technics of perfusion would enhance the effectiveness of the alkylating agents by permitting administration of larger doses and through the production of high oxygen tensions within the tumor-bearing tissues. It would be presumptious indeed to attempt to evaluate the results on the basis of length of survival or relief of symptoms since a majority of patients were incurable and none have been followed for more than a year after treatment. On the other hand, if the effect of perfusion on the tumor is used as a criterion, then treatment was successful in 18 of the 19

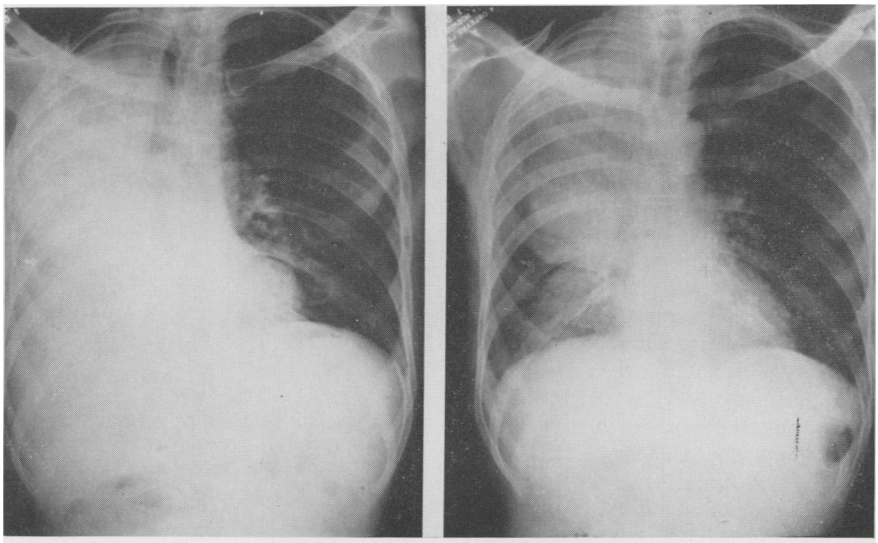

FIG. 12. Roentgenograms of the chest in Case 4, (a) before, and (b) after perfusion.

cases followed long enough for changes to be evident. That is, in 18 patients there was gross and/or microscopic evidence of significant tumor necrosis, occurring, in many instances, in neoplasms heretofore believed to be resistant to the mustard compounds.

Phenylalanine mustard was employed in the treatment of metastatic melanoma in humans by Hales [8] and Stehlin [14] following the demonstration by Luck [11] that this agent produces regression of transplantable melanomas in rats. Although a decrease in size of some of the lesions was noted in no instance was regression sufficient to suggest that the tumor was controlled. Therefore, the response to perfusion with PAM observed in case one was unanticipated. There is little doubt that the lesion has been controlled for about ten months. During the interval since perfusion progressive regression and disappearance of metastases have occurred, no new lesions have formed and none of the original tumors has increased in size. Experience with four additional patients with malignant melanoma has been encouraging also and suggests that the regressive changes occurring in these melanomas are characteristic and are indicative of the specificity of phenylalanine mustard for this tumor.

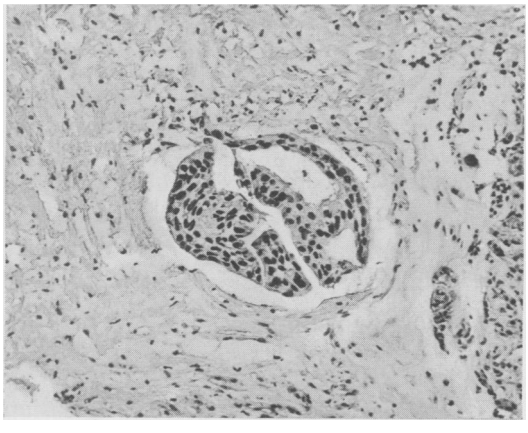

FIG. 13. Photomicrograph of section removed from the right main bronchus prior to perfusion in Case 4.

The only operative death in the series occurred after perfusion of a lower extremity extensively involved with metastatic melanoma. The patient had a primary lesion on the left leg excised and this was followed by groin dissection. Within a short time metastases appeared on the en-

tire limb, in the abdominal wall and in the iliac nodes. Because of the widespread lesions, perfusion was carried out through the abdomen utilizing the aorta and vena cava. A dosage schedule of 3 mg./Kg. body weight was employed rather than the usual dosage of 2 mg./Kg. Dye studies revealed that isolation was not complete and although about 1,500 ml. of blood was removed from the vena cava after perfusion, hematologic changes developed and the patient died on the fifteenth day of severe bone marrow depression. This case emphasizes the necessity for isolation of a perfused vascular bed if toxic effects are to be avoided. There have been two late deaths also, occurring in patients whose neoplasms were hopelessly advanced at the time of perfusion.

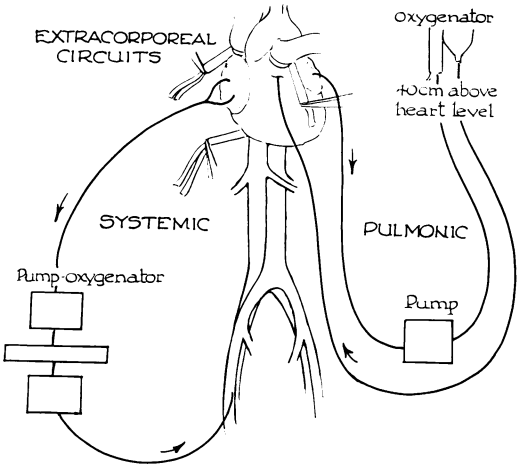

FIG. 14. Technics of cannulation for perfusion of the lungs. Two extracorporeal circuits are required in order to completely isolate the lungs.

The response of rhabdomyosarcoma to perfusion with nitrogen mustard is equally striking. Regressive changes were evident within 24 hours after perfusion and continued for about three or four weeks. Microscopically these changes consisted of extensive coagulation necrosis of tumor cells with round cell infiltration and fibrous connective tissue replacement. The bulk of these tumors alone probably eliminated the possibility of a cure but the remarkable response to perfusion suggests that this modality may be extremely useful as adjunctive therapy. In one of the patients with osteogenic sarcoma there was questionable evidence that the tumor was actually disseminated within the extremity by perfusion. This was an extensive, rapidly growing lesion and perhaps treatment should not have been undertaken. In one other patient with osteogenic sarcoma perfusion with nitrogen mustard produced significant reduction in the size of the lesion which involved the shoulder and microscopic examination revealed tumor necrosis. Forequarter amputation subsequently was performed. There was one case of chondrosarcoma treated by perfusion with PAM. Biopsy of the lesion after operation revealed extensive destruction of the tumor and its size was reduced by about one-third. Amputation of the involved extremity (upper) was advised but refused. In four patients with localized sarcomas of the lower extremity perfusion was carried out prior to excision. Each of these lesions showed histologic evidence of cell necrosis but insufficient time has elapsed to determine whether perfusion with the mustard compounds will alter the usually poor prognosis for these tumors.

Among the cases of carcinoma only two were primarily resectable and chemotherapy was considered to be adjunctive. In one of these a carcinoma of the transverse colon was isolated and perfused with Chlorpactin XCB® just prior to removal. In the other, an epidermoid carcinoma of the thumb was perfused with nitrogen mustard and the involved digit removed two weeks later. At the time of excision the lesion had decreased in size by about one-half and biopsy disclosed necrosis of neoplastic cells.

A patient with carcinoma of the breast received treatment for a lesion that was considered to be inoperable because of its large size, local extension and extensive axillary metastases. Following perfusion

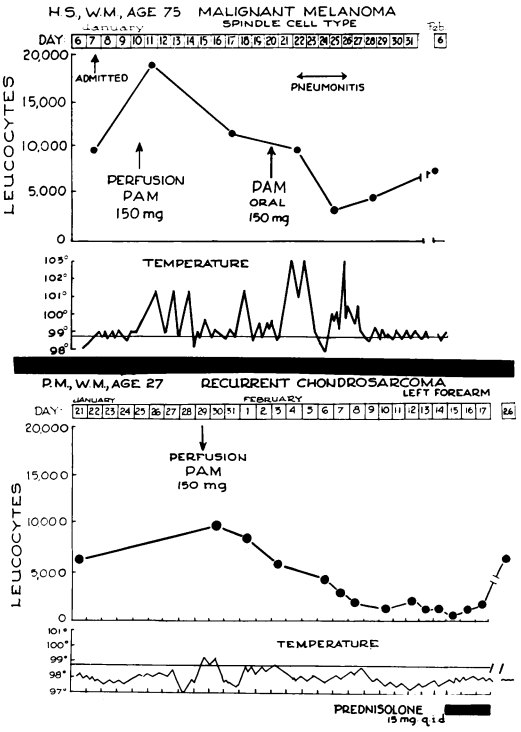

CHART 2 (Upper). The difference in leucocyte response to PAM administered by perfusion and systemically is demonstrated in this case. The patient had extensive cutaneous metastases and a solitary pulmonary metastasis arising in a malignant melanoma of the left leg. The extremity was perfused without significant change in the leucocyte count but following oral administration of PAM the leucocyte count was severely decreased.

CHART 3 (Lower). Following perfusion of the left upper extremity with PAM in a patient with chondosarcoma, the tourniquet was released without first draining the extremity. About the sixth day after perfusion the leucocyte count began to fall, reaching a low of 500 cells per cu. mm. on the seventeenth day.

with nitrogen mustard there was sufficient regression both in the primary tumor and in its metastases to permit radical mastectomy.

Perfusion of carcinoma of the lung with nitrogen mustard was undertaken primarily to determine whether isolated perfusion of the lungs was possible. The technic employed appeared satisfactory although there was only moderate change in the neoplasm. The remaining cases of carcinoma consisted of lesions that involved pelvic structures and in each instance therapy was palliative only. In only one case, described above, has sufficient time elapsed for evaluation of therapy and in this instance necrosis of a large pelvic metastasis demonstrates the cancerocidal effects of nitrogen mustard when administered in high concentrations. In all of the cases of pelvic carcinoma, isolation was possible and no significant systemic toxicity was noted.

The increased cytotoxic effects of the mustard compounds observed in this study are primarily a result of the high concentrations achieved by perfusion and confirm the observations of Klopp [10] regarding intra-arterial administration. Perhaps much larger total doses could have been employed since it was demonstrated in animal experiments that as much as 0.8 mg./Kg. of nitrogen mustard can be administered by perfusion without injury to normal tissues. It is not certain whether maintenance of a high oxygen tension actually increases the effectiveness of nitrogen and phenylalanine mustard. As indicated earlier, the effect of ionizing radiation is enhanced by high oxygen tensions and since the alkylating agents act upon the cell in a manner similar to radiation, it may be assumed that this state, if maintained during perfusion, will potentiate the action of these compounds.

Biochemical determinations performed on blood from the perfused and systemic circuits in one case failed to demonstrate any significant alteration. These studies are being continued.

Perhaps the most significant feature of this study is the demonstration that certain anatomic areas can be isolated from the systemic circulation with relative ease and perfused for periods up to 30 minutes without mixing between the systemic and perfused circuits (Chart 2). Certain precautions must be taken, however, when PAM is employed, to be certain that isolation is complete and that the area is drained of

contaminated blood upon completion of perfusion. Failure to do so may result in severe systemic toxic effects due to the long duration of activity of PAM (Chart 3). These technics of isolation open the way to the use not only of agents with greater cancericidal, albeit greater hematologic, effect than the mustards, but to the use of agents for the treatment of infection as well. For instance, antibiotic agents that are highly bacteriocidal but exert a toxic effect on certain viscera, might be administered by perfusion for the treatment of refractomy osteomyetitis in the limbs, or persistent chronic pulmonary infections might be treated with specific agents administered solely to the lungs by perfusion.

Although there are many things pertaining to the methods described above which require further investigation, it appears that the technics of perfusion are valuable in the chemotherapy of cancer. By the use of these technics a high concentration of the agent can be maintained within the tumor-bearing area without risk of producing systemic toxic effects. Thus, there is no interference with hemopoiesis and the host antibody response to the neoplasm is preserved. The ability to remove contaminated blood from the part after perfusion further protects against toxic effects when long acting agents are employed.

On the basis of preliminary clinical experience it is concluded that the administration of chemotherapeutic agents by perfusion is useful as adjunctive therapy for control of localized malignant tumors and for palliation of certain far-advanced lesions.

Summary

1. Chemotherapy of cancer has not been entirely satisfactory because the administration of doses large enough to significantly affect a tumor produce serious toxic effects on the bone marrow and gastrointestinal tract.

2. Technics have been developed for isolating and perfusing a tumor-bearing area with chemotherapeutic agents, utilizing a heart-lung apparatus.

3. Experimental studies demonstrated that by these technics the limbs, intestine, liver, pelvis, and lungs can be isolated from the systemic circulation and perfused for periods up to 30 minutes.

4. Employing these technics 24 patients with malignant neoplasms have been treated. The results suggest that these methods are valuable as adjunctive therapy for localized tumors and for palliation of far-advanced lesions.

Bibliography

1. Bateman, Jeanne C., S. T. Klopp and J. K. Cromer: Hematologic Effects of Regional Nitrogen Mustard Therapy. Blood, **6**:26, 1951.
2. Churchill-Davidson, I., C. Sanger and R. H. Thomlinson: Clinical Application: in Oxygenation in Radiotherapy Symposium. Brit. J. Radiology, **30**:406, 1957.
3. Craver, Lloyd F.: The Nitrogen Mustards: Clinical Use. Radiology, **50**:486, 1948.
4. Dougherty, T., A. Gilman, L. Goodman and J. Dougherty: Cited by Gilman & Philips.
5. Gilman, Alfred and F. S. Philips. The Biologic Actions and Therapeutic Applications of the B-Chloroethyl Amines and Sulfides. Science, **103**:409, 1946.
6. Gilman, A., L. Goodman, G. E. Lindskog and J. Dougherty: Cited by Gilman & Philips.
7. Gray, L. H.: Radiobiological Considerations: in Oxygenation in Radiotherapy—Symposium. Brit. J. Radiology, **30**:403, 1957.
8. Hales, D. R., B. E. Hall, J. Franco, R. W. Jerner, F. M. Willett and T. V. Feichtmeir: p-Di (2-Chloroethyl) Amino-L-Phenylalanine in the Treatment of Metastatic Melanoma. Proc. Am. A. Cancer Res., **2**:210, 1957.
9. Klopp, C. T.: Regional Intra-Arterial Nitrogen Mustard as an Adjunct to Radiation Therapy. Am. J. Roentgenol., **70**:1005, 1953.
10. Klopp, C. T., T. C. Alford, J. Bateman, G. N. Berry and T. Winship: Fractionated Intra-Arterial Cancer Chemotherapy with Methyl Bis Amine Hydrochloride. Ann. Surg., **132**: 811, 1950.
11. Luck, J. Murray: Action of p-Di (2-Chloroethyl)-amino-L-phenylalanine on Harding-

Passey Mouse Melanoma. Science, 123:984, 1956.

12. Ryan, R. F., E. T. Krementz, O. Creech, J. N. Winblad, W. Chamblee and H. Cheek: Selected Perfusion of Isolated Viscera with Chemotherapeutic Agents. Surgical Forum, 8:158, 1958.

13. Ryan, R. F., J. N. Winblad, E. T. Krementz and O. Creech: Treatment of Malignant Neoplasms with Chemotherapeutic Agents Utilizing a Pump-Oxygenator: Technics and Early Results. Bull. Tulane Univ., 17:133, 1958.

14. Stehlin, John: Personal Communication.

Discussion

Dr. Warren H. Cole: I think this is a very clever idea, and I want to congratulate the authors. This modification of the use of alkylating agents represents a definite improvement over the conventional method of administration because the toxicity of most of these agents is so high.

It's been a bit discouraging during the past 10 or 12 years to realize that perhaps as many as 50,000 chemicals have been screened for effect against cancer, yet none of them seems to be any more effective than the original one, nitrogen mustard. We were hoping that as these numerous agents were tried we would find one acting like the antibiotic penicillin, namely, that you could give it in huge doses without toxicity. However, we have not found such an agent. All of the alkylating agents are quite toxic. Accordingly, I believe we must give serious thought to methods of this sort, hoping to utilize them as the primary agent in destroying local tumors until we get better drugs.

There is one disadvantage, of course, which the authors admitted; namely, they are treating the tumor locally. Treatment of metastases by this method is not practical.

However, I hope these authors will continue with this work (and I know they will) because as long as the tumor remains local, even though it's very massive, we may be able to control it.

Dr. John Schilling: I rise to congratulate Dr. Creech on some unusual observations. This work indicates the productivity of poverty, from which we should all, perhaps, draw an analogy.

Did you evaluate microscopically the effects of the regional perfusion on normal structures?

And, secondly, have you perfused the liver, with observations of normal structure and damage, as well as effect on metastases?

Dr. Denton A. Cooley: We have also been conducting some experiments using temporary extracorporeal circulation for reasons other than cardiac surgery. Our idea was that the extracorporeal circuit containing the stream of blood could be exposed to strong irradiation without exposing the patient's body. This work was done in collaboration with Drs. Vincent Collins and George Morris.

A simple circuit was devised to remove blood from a femoral artery and pump it into a plastic bottle from which the blood was returned to a femoral vein. The bottle was placed inside of the tube of a 2 million volt Van de Graaff electrostatic generator. During the irradiation of blood the animals were shielded from the irradiation.

Dogs were used for the experimental animals and periods of extracorporeal irradiation up to 65 minutes were tried. Total irradiation was between 21,000 and 9,600 roentgens.

After many experiments we were somewhat surprised to discover that red blood cell counts increased after the perfusion, the maximum response being 2 to 3 months later. We had actually expected to find a decrease in blood count if anything.

Our original hope was that this method would have some usefulness in treatment of blood dyscrasias or leukemias. Irradiation of the blood alone would avoid exposure of the blood forming organs of the body. Destruction of a large number of leukemia cells might have some favorable effect on this disease in stimulating defense mechanisms in the body. In one terminal patient with chronic myelogenic leukemia this method was tried, but this 72-year-old patient expired 3 days later with coronary occlusion before studies would be completed. Further work on the problem of extracorporeal irradiation should be done in order to determine whether any benefit could be obtained in cancer patients.

Dr. Oscar Creech, Jr. (closing): I wish to thank Drs. Cole, Schilling and Cooley for their remarks.

In reply to Dr. Schilling's question, we have noted no ill effects on normal tissue after perfusion. In some instances, normal muscle becomes edematous but there is no apparent cellular damage and edema subsides rapidly.

We have not had a patient whom we consider suitable for perfusion of the liver, although technics have been developed for perfusing this organ.